VOL. VII, No. 9 NOVEMBER, 1946

BORDEN'S REVIEW *of* NUTRITION RESEARCH

EXPERIMENTAL VITAMIN DEFICIENCY IN MAN

BORDEN'S REVIEW
of
NUTRITION RESEARCH

Reviews and summaries of research information concerning nutrition and foods. Published monthly, except July and August, by THE BORDEN COMPANY; officers of the Company: Theodore G. Montague, president; E. L. Noetzel, treasurer; T. D. Waibel, secretary. Editorial office: 350 Madison Avenue, New York 17, N. Y.

EXPERIMENTAL VITAMIN DEFICIENCY IN MAN

By **RUTH WOODS**

Without the laboratory animal there would be no science of nutrition as it is known today. The discovery of the individual nutritive essentials of the diet as well as an understanding of the role which these essentials play in the complex processes of body metabolism are based largely on experiments with animals. That much of this work has been almost directly translatable into terms of the human economy is often casually taken for granted. Yet, marked differences often do occur in the biological responses of different species of animals subjected to identical test conditions. In view of the existence of such species differences, it is necessary to have experimental nutrition studies conducted directly with humans.

Within the past ten years many such studies have been undertaken and, as may be expected, have served to bridge the gap between the findings of animal experimentation and their extension to human biology. These studies have contributed to a more precise knowledge of the manifestations of human avitaminoses. Furthermore, they have served as a basis for determining human vitamin requirements and have led to the development of valuable diagnostic methods for detecting early signs of vitamin deficiency. The great majority of these studies has been concerned with deficiencies of the B vitamins, and because the scope of this subject is so large, the present Review will be limited to studies with members of the vitamin B group.

Historical Background:

Investigation into experimental vitamin deficiency in man represents a large field of activity in nutrition research, dating back to the original discovery of the vitamins. Thus, no sooner had polyneuritis in chicks been traced to the absence of a dietary factor in polished rice, than experiments were undertaken by the Japanese navy in an effort to relate beri-beri — the human analog of the disease — to the same cause. Likewise, the observation that lime juice (actually lemon juice) could cure scurvy — the inevitable sailor's disease — was a contribution of the British navy to the discovery of vitamin C. Goldberger's classic prison farm experiments led to the establishment of pellagra as a dietary deficiency disease. The exportation of butter from Denmark during the first World War unwittingly proved to be the confirmation of animal studies on the human need for the new "fat-soluble vitamine A," when Danish children, deprived of this food, developed the eye disease xerophthalmia. The realization that rickets could be prevented

by the simple expedient of exposure to sunshine as a source of vitamin D or by the administration of the vitamin in fish liver oils, completed the early background of observations on disturbances induced in man as a result of dietary inadequacies. These studies, in the main, either contributed to the discovery of the vitamins or served to confirm early animal experiments indicating a need for certain additional dietary factors beyond proteins, fats and carbohydrates in human diets.

With the discovery of the vitamins and their subsequent availability as natural concentrates or synthetic compounds, experimentation with human subjects took on new meaning and scope. It became possible to prepare diets with vitamin contents which were more precisely known and which could be altered according to controlled plans. These studies in which single vitamins were partly or completely omitted from human experimental diets led to a number of new fundamental concepts in nutrition. The most outstanding of these was the realization that the so-called "classic" deficiencies — pellagra, beri-beri and scurvy — as observed in man, are manifestations of *multiple* vitamin deficiencies rather than of a single vitamin deficiency. In human beings, "pure" deficiency diseases are rare because people on inadequate diets generally are deficient in several essential nutrients. The use of human experimental subjects in the production of *isolated* vitamin deficiencies demonstrated the fact, for example, that only certain symptoms of clinical pellagra are induced by a lack of nicotinic acid in the diet. Thus human pellagra as it occurs among ill-fed people presents a multiplicity of symptoms which include not only the dermatitis of nicotinic acid deficiency, but often the mental and nervous disturbances of thiamine deficiency, the anemia of folic acid deficiency, the eye and mouth ailments of riboflavin deficiency and even the scorbutic signs of vitamin C deficiency.

Another major contribution of human studies with isolated vitamin deficiencies is the recognition that vitamin deficiency may exist in a mild or sub-clinical but still serious form long before evidence of the classic deficiency diseases becomes apparent. This has represented a significant advance not only in nutrition, but in modern clinical medicine. Many hitherto inexplicable disturbances of the various organs or physiological systems of the body can now be traced to vitamin inadequacy and relieved by vitamin therapy.

Early Studies:

Among the earliest controlled experiments in the deliberate induction of a vitamin deficiency in a human being were those reported in 1935 by Katherine Elsom (1) of the Hospital of the University of Pennsylvania. At that time the multiple nature of the vitamin B complex was but newly recog-

nized and only a few of its presently known components identified. Not much was known concerning the milder or sub-clinical forms of vitamin deficiency. As Dr. Elsom pointed out, "clinical disturbances incident to mild deficiency of vitamin B in the diet, as opposed to frank deficiencies such as beri-beri and pellagra, are commonly believed to be very rare in man and are thought to occur only when the diet is grossly abnormal or when there is serious interference with absorption from the gastrointestinal tract." That such subclinical manifestations of vitamin deficiency were not at all as rare as was supposed, however, was strongly suspected by Elsom as a result of observations on numerous patients on diets which were not obviously abnormal. As a test of this hypothesis, two patients were selected for feeding experiments in which the vitamin B content of the diet was deliberately restricted. The resulting deficiency symptoms were carefully noted and recorded.

One patient, formerly suffering from malnutrition, but apparently in good health at the start of the experiment, was placed on a diet moderately deficient in "vitamin B" for five months. When clinical manifestations of deficiency became apparent, various fractions of the vitamin B complex were added to the diet and the results of their administration noted. In the case of the second patient, who had entered the hospital with fully developed signs of vitamin deficiency, the diet was also restricted in its vitamin B content and the effects of subsequent vitamin supplements noted. The first patient developed symptoms of deficiency after five weeks on the experimental diet. In both patients these symptoms included, among others, loss of weight, edema, lowered blood pressure, loss of gastrointestinal tone, and loss of appetite. In both cases administration of vitamins was followed by weight gains even in the face of loss of considerable edema fluid from the body. Disappearance of edema was associated with a restoration of normal serum protein levels and the re-establishment of a positive nitrogen balance. Vitamin B_1 and B_2 concentrates were effective in partly restoring normal body status, but it was found that complete cures could be effected only by administration of yeast as well. As in the classical animal experiments, this indicated that human beings required additional factors of the B complex, at that time unknown.

By the following year, 1936, the availability of vitamin B_1 in pure crystalline form (2) made possible more precise control of human experimental diets. This was soon followed by the synthesis of nicotinic acid and riboflavin, two members of the "vitamin B_2 complex." In 1939, Spies, Bean and Ashe reported spectacular improvements in severely malnourished patients receiving synthetic B vitamins as the only supplements to their usual inadequate diets (3). The absence of complete restoration to health, how-

ever, again indicated the need for still other as yet unidentified vitamin factors. At the same time, it was pointed out that human vitamin deficiency diseases are not usually due to the lack of a single vitamin, but rather are the result of multiple deficiencies. Thus, for example, it is generally accepted at the present time that pellagra — primarily a manifestation of nicotinic acid deficiency — has certain aspects which are purely the result of a lack of thiamine or of riboflavin and will, therefore, not respond to nicotinic acid therapy alone.

Studies of Isolated Vitamin Deficiencies:

Since human beings on ordinary mixed diets rarely, if ever, develop deficiency symptoms attributable to the lack of a single vitamin, the experimental induction of a single vitamin deficiency state in an otherwise normal human subject presented itself as a highly desirable line of investigation.

Thiamine: Early in 1939, Jollife, Goodhart, Gennis and Cline (4) reported the results of just such a study. They selected five young internes at Bellevue Hospital as experimental subjects. The men received experimental diets adequate in all known essentials except thiamine. These diets provided 0.473 milligram of thiamine per subject per day, which is less than one-half of the minimum adult requirement (1.0 mg. per day) and less than one-third of the National Research Council's recommended allowance (1.5 mg. per day). It was surprising to find that symptoms and objective evidence of deficiency appeared as soon as four or five days after the start of the test period. One of the five subjects, however, remained completely symptom-free throughout the 30-day test period.

Soon after starting on the thiamine-deficient diet, the subjects began to complain of loss of appetite, pain in the pit of the stomach, burning of the feet, shortness of breath on exertion, muscle cramps and palpitation. Shortly afterward, objective signs were noted: Extreme sensitivity of the skin to pain stimuli, changes in electrocardiogram readings and tenderness of the calf muscles. The daily addition of 3.83 mg. of thiamine without knowledge on the part of the subjects caused all subjective symptoms to disappear within three days and all objective signs within six days.

At about the same time these studies were in progress, similar investigations were being conducted at the Mayo Clinic by Williams, Mason, Wilder and Smith (5, 6). In their preliminary report the first three of these workers reported their observations on four young women who had been placed on a diet deficient in thiamine for 21 weeks. Despite this restriction, the subjects failed to exhibit the classic symptoms of beri-beri, showing only mild signs of nutritional failure. Accordingly, the experiment was repeated in greater

detail with new subjects. The subjects were female inmates of the Rochester State Hospital who had "recovered" from acute phases of mental illness and who were fully capable of exercising decision in volunteering for the experiments. The patients were excellent subjects because they were accustomed to confinement, willing to cooperate and, because of the circumstances, automatically served as their own controls in the pre- and post-experimental periods.

The second study confirmed the earlier observations that isolated withdrawal of thiamine from the diet does not produce beri-beri, even though thiamine restriction in the Rochester group was more severe (0.15 mg. per patient per day). The symptoms exhibited were similar to those observed by Jolliffe. In addition, periodic determinations were made of body weight, physical and neurological signs, tolerance for exercise, gastric analysis, blood analyses, sugar tolerance, basal metabolism. Early symptoms noted after several weeks on the thiamine-deficient diet were mental depression, dizziness, fatigue, backache, loss of weight and appetite, insomnia, nausea, vomiting, impaired gastrointestinal motility, decreased activity and capacity for work. Edema did not occur nor did changes in plasma protein concentration. It is interesting to note that although these women received only about one-third the amount of thiamine allowed the male subjects at Bellevue, they manifested symptoms much later. This may have been due to somewhat lower requirements on the basis of sex and activity.

The results of thiamine administration were remarkable. As described by the authors:

> "Subjective improvement was observable in every case within a few hours after the initial injection of 1 mg. of thiamine hydrochloride. Nausea and vomiting ceased; food which previously had been revolting . . . was eaten without urging; fatigue disappeared; activity was resumed and apathy was replaced by lively interest in ward work and current events. During the 18 days in which administration of thiamine hydrochloride represented the only change made all signs and symptoms incident to the period of restriction of thiamine disappeared. The electrocardiograms became normal; the previously diabetic type of sugar tolerance curve was replaced by a normal curve; . . . previous sluggish motility of the intestinal tract was replaced by normal activity."

These observations were confirmed in subsequent studies by the Mayo Clinic group (7, 8, 9) even in tests with only moderate thiamine restrictions as might occur on ordinary mildly inadequate diets. In one of these studies, (9), Williams and co-workers were able to demonstrate for the first time that the polyneuritis of beri-beri is actually caused by lack of thiamine rather than of any of the other B vitamins. There had been some doubt of this because in earlier human experiments polyneuritis failed to develop, whereas neurological manifestations had been observed in many experimental animals

on diets deficient in riboflavin, pyridoxine or pantothenic acid. In their earlier studies, Williams *et al* had maintained their subjects on a diet providing either 0.075 or 0.22 mg. of thiamine per 1000 calories per day. The former allowance provided so severe a restriction that the experiment had to be discontinued because of prostration and inanition just when neuropathy seemed certain of development. The more moderate restriction, on the other hand, caused general impairment of mental and physical health with no detectable involvement of peripheral nerves. A diet providing a thiamine restriction intermediate between these two levels was, as anticipated, successful in causing such symptoms to appear. Only two subjects were used in this experiment. Both developed signs of nerve involvement in the extremities within the 120-day test period as evidenced by tenderness of calf muscles, depression or disappearance of the tendon reflexes and paralysis of the muscles of the thighs and legs. It was concluded that polyneuritis is a manifestation of severe thiamine deficiency as opposed to the generalized symptoms previously described as signs of mild thiamine deficiency.

(Implications of Thiamine Studies): It is apparent, from a survey of the human experimental studies on induced thiamine deficiency that a whole series of physical and nervous disturbances may result from a thiamine intake of less than one milligram per day. While extensive dietary surveys seldom have shown intakes of thiamine as low as those used to induce severe experimental deficiencies, a large proportion of families in this country have diets providing less than the recommended allowance of 1.5 mg. per day.*

An interesting experiment by Najjar and Holt (10) was reported in 1943. These workers, in an attempt to extend the observations of induced thiamine deficiency in man, encountered some unexpected results which led not only to a possible explanation of apparent discrepancies among earlier workers, but to the development of a new field of investigation — the biosynthesis of vitamins by human beings.† In their studies, nine adolescent male volunteers were given gradually reduced thiamine intakes over a period of many months. Eventually, after an intake of from 0.1 to 0.2 mg. per day for months, thiamine was omitted completely from the diet. Four of the nine subjects then showed no signs of deficiency during a subsequent observation period of seven weeks. In spite of the fact that the diet contained no thiamine whatsoever, each of these subjects had large quantities of free thiamine in the feces. The source of this thiamine was convincingly traced to synthesis in the body by intestinal bacteria. In subsequent studies (11), the possibility of similar synthesis of riboflavin was discussed by these work-

---

*cf.—*Borden's Review of Nutrition Research,* May, 1944—"The Prevalence of Malnutrition."
†cf.—*Borden's Review of Nutrition Research,* May, 1946—"The Biosynthesis of Vitamins."

ers. In the light of these observations, new consideration must be given to the estimation of human requirements as influenced by the phenomenon of intestinal biosynthesis. The possibility also exists that individuals may vary greatly in their ability to withstand dietary deprivation of vitamins, depending upon their individual biosynthetic activities.

In addition to the physical and functional disturbances associated with thiamine deficiency, considerable evidence of mental disturbance is noted. Williams *et al* (6) and other investigators have remarked on the similarity between the mental symptoms of thiamine deficiency and the psychiatric syndrome designated as neurasthenia.* Bueding *et al* (12), in similar studies, have pointed out that in all their cases of induced thiamine deficiency, mental and physical inefficiency preceded, by weeks or months, other more objective signs of thiamine deficiency.

Riboflavin: Early in the history of pellagra many writers noted that the disease sometimes occurred without the characteristic skin lesions which gave it its name. The term "pellagra sine pellagra" (i.e. pellagra without 'pelle agra', or rough skin) was introduced to describe this form of atypical pellagra. Prominent among the symptoms observed in this condition were certain types of cracks and fissures in the corners of the mouth, reddening of the lips and seborrhea about the nose. As early as 1918, Goldberger and his associates (13) suggested that two different dietary factors might be involved in pellagra. Following the many observations along these lines, it was finally suggested by Sebrell (14) in 1938 that clinical pellagra might be a multiple deficiency. He pointed out that a deficiency of riboflavin might occur simultaneously with, or even independently of pellagra. In order to determine whether the condition known as pellagra sine pellagra is due to a deficiency of nicotinic acid or some other vitamin, a series of experiments with human subjects was undertaken by Sebrell and Butler (15, 16).

In one study (17) eighteen adult women in an institution were selected as subjects after careful examination had revealed them to be in good physical health. The diet of the women was changed from the general, varied institution diet to a specially devised modification of the Goldberger-Tanner pellagra-producing diet which is low in nicotinic acid and almost free of riboflavin. The diet was carefully weighed during preparation and in serving. The subjects all ate together under supervision and any food left over on the plates at the end of the meal was weighed so that an accurate estimate of the actual food intake could be obtained. After three to four months on this ration, ten of the eighteen women developed lesions similar to those de-

*The nervous and mental disorders directly associated with vitamin deficiency will be discussed in greater detail in a future issue of this *Review*.

scribed as pellagra sine pellagra. These signs were described as a "reddened, denuded lesion of the lips, maceration and fissuring in the angles of the mouth and seborrheic accumulations at the nasolabial folds." The term *cheilosis* (morbid condition of the lips) was adopted as a collective name to describe these symptoms. Prior to this time one of the subjects exhibited mild skin lesions of pellagra which responded completely to nicotinic acid therapy. Cheilosis appeared several weeks after the healing of the skin lesions and, despite increasing doses of nicotinic acid, persisted with increasing severity for almost two months. Administration of riboflavin at this time caused the complete disappearance of the mouth lesions within six days. Similar results were obtained with the other subjects. Discontinuance of the daily riboflavin supplement resulted in a recurrence of the cheilosis which responded again promptly when riboflavin was restored. Nicotinic acid had no effect on the cheilosis.

On the 139th day of the experiment the eight subjects who had not yet manifested symptoms of cheilosis were divided into two equal groups. One of these continued on the deficient diet while the other group received a daily preventive dose of riboflavin. Three of the four non-protected patients eventually showed cheilosis, while none of the protected group showed lesions of any kind during the entire 365-day period of observation.

From these observations it was concluded that the lesions formerly called pellagra sine pellagra are actually manifestations of riboflavin deficiency and constitute a clinical entity in themselves, now named *ariboflavinosis.* Additional clinical observations by these and other investigators have since demonstrated that ariboflavinosis may be a common complication of pellagra as well as an isolated deficiency condition (3, 17, 18, 19, 20, 21, 22). As a result of these further studies, additional clinical symptoms of ariboflavinosis were soon recognized. Prominent among these are various eye disturbances such as conjunctivitis, tearing and burning of the eyes and failing vision which were first reported by Spies *et al* (23) to respond to riboflavin therapy.

Nicotinic Acid: The studies on nicotinic acid deficiency in human subjects have already been described in great detail in previous issues of this Review.* More recent investigations at the Nutrition Clinic of the Hillman Hospital in Alabama and at the Mayo Clinic in Rochester, as well as elsewhere have served to clarify the symptoms of nicotinic acid deficiency and to separate them from symptoms associated with pellagra, but due primarily to the lack of other vitamins.

Mixed Vitamins: In an experiment by Barborka *et al* (24), four medical

*cf.—*Borden's Review of Nutrition Research,* October, 1943 and November, 1943, "The Conquest of Pellagra" and "Clinical Studies With Nicotinic Acid."

students were maintained on a mildly vitamin B-complex deficient diet containing 0.65 mg. of thiamine and 0.94 mg. of riboflavin. No objective evidence of vitamin deficiency was observed, but subjective symptoms such as fatigue, loss of appetite, decreased performance on a bicycle ergometer and generally lessened work output were noted. Supplementation of the diet with vitamin B complex resulted in a restoration of work output to efficient levels. Similar reports were announced by Egaña *et al* (25) at the Harvard Fatigue Laboratory where studies were conducted on seven men on diets low in the entire vitamin B complex.

During the recent war, Keys and associates (26, 27, 28, 29, 30) conducted an extended series of studies on the relation in normal men between intake of vitamins of the B complex and various aspects of "fitness." As a measurement of fitness they tested work performance and capacity, cardiovascular, sensory and psychomotor functions and aspects of intermediary metabolism. Measurements of intellectual and other purely psychological factors were also included. The subjects included university students, soldiers in the U. S. Army and conscientious objectors. In the early studies it was found that addition of vitamin B complex to adequate diets, such as the Army ration, did not increase capacity for hard, muscular work and that existence on a mildly deficient diet did not impair such capacity. In direct conflict with these observations were reports by Johnson *et al* (3) that such deprivation of B vitamins markedly impaired the ability to perform physical labor. The discrepancy in results of the two groups was thought to be due chiefly to differences in the conditions of the tests. In their latest study, Keys *et al* (29) succeeded in obtaining definite deficiency symptoms in a group of conscientious objectors receiving a diet deficient in all of the B complex. Of the several vitamins under investigation, thiamine deficiency was most pronounced.

Significance of Human Studies:

The studies of experimentally induced isolated vitamin deficiencies described in this paper have contributed three major advances to nutrition research which could not have been achieved through animal studies. First has been the recognition of the symptoms — subjective and functional — which may be expected to result from a mild deficiency of one or more of the vitamins. The recognition of such early signs of vitamin deficiency make possible early treatment and the subsequent prevention of more severe forms of malnutrition. Thus, vague symptoms of weakness, fatigue, headache, failing vision, burning of the eyes and abnormal sensitivity to light, lack of appetite, nausea, poor memory, mild mental depression, skin lesions and unusual reddening of the lips or tongue, once formerly discarded as insigni-

ficant or largely the result of imagination must now be looked upon as possible signposts of beginning vitamin deficiency. That these symptoms may have other causes as well must not be lost sight of. But in the absence of any obvious cause their appearance must always suggest further checking of the nutritional status of the individual.

This brings up the second contribution of human nutrition studies — the development of valuable objective diagnostic methods for confirming suspicion of nutritional inadequacy. Two interesting examples of this are found in electrocardiogram responses and in observations on gastrointestinal motility. Cardiologists who normally disregard slight variations in characteristic wave patterns as evidence of cardiac disturbance now realize that certain of these minor deviations are specific early indications of thiamine deficiency. Likewise, in the absence of any gross structural abnormality, slightly delayed excretion of a barium test meal may be disregarded as evidence of intestinal obstruction, but must now be recognized as another sign characteristic of thiamine deficiency. Biomicroscopic examination of the eye will confirm suspicion of vitamin A and riboflavin deficiency through recognition of characteristic changes in the cornea. These diagnostic methods serve the dual purpose of confirming vitamin deficiency as evidenced by vague subjective symptoms, as well as of providing routine clinical methods for early detection of such deficiency even in the absence of subjective complaints. An outline of these diagnostic procedures made possible by observations on human subjects has been presented in an earlier issue of this Review.*

A final major contribution of human studies has been the establishment, within relatively precise boundaries, of the human requirement for many of the vitamins under varying conditions of age, sex, activity and environmental surroundings. While such values theoretically might be, and often have been, deduced by relative comparison with animal requirements on a body weight basis, the figures thus obtained are at best only approximations which do not take into account differences in species response, need and utilization of the various nutrients and their possible biosynthesis. Many of the experiments on induced vitamin deficiency in man have included observations on urinary output of the vitamins as affected by different, measured levels of vitamin intake, thereby serving as important balance studies. Correlation of vitamin intake levels with the appearance of early deficiency signs and quantitative determination of the amounts of vitamin supplements required to overcome these deficiency manifestations have provided a means for determining minimum protective requirements of many of the vitamins.

**cf.—Borden's Review of Nutrition Research,* February, 1945, "Diagnosing Nutritional Deficiencies."

Conclusion:

Within the past ten years the science of nutrition has made tremendous strides. From a vague, general science delineating the gross dietary components necessary to good health and indicating the need for minute, more or less unknown "vitamine" constituents, nutrition science has developed to the point where almost all of the vitamins have been unearthed, chemically defined and their physiological requirements — both functional and quantitative — established. The great bulk of this information has been derived from countless painstaking experiments with animals. Equally important contributions have come from the laboratories of organic chemists. For the ultimate certainty of nutrition principles as applied to practical human problems, however, the outstanding contributions of human studies with volunteer subjects must be acknowledged. Human experimental studies have led to the development of the multiple-deficiency concept; to the recognition of new and hitherto unsuspected avitaminosis syndromes; to precise methods for early detection of vitamin deficiency; to the establishment of a rational basis for vitamin therapy; and to the evolution of vitamin requirement standards. Through this medium a clearer insight has been obtained into the significance of vitamin factors in health and disease than could be afforded from clinical observation and animal experimentation alone.

REFERENCES CITED

1. ELSOM, K. Experimental study of clinical vitamin B deficiency. *J. Clin. Invest. 14* (1935).

2. WILLIAMS, R. R. Structure of vitamin B_1. *J. Amer. Chem. Soc. 58,* 1063 (1936).

3. SPIES, T. D., W. B. Bean and W. F. Ashe. Recent advances in the treatment of pellagra and associated deficiencies. *Ann. Intern. Med. 12,* 1830 (1939).

4. JOLLIFFE, N., R. Goodhart, J. Gennis and J. Cline. The experimental production of vitamin B_1 deficiency in normal subjects. *Am. J. Med. Sci. 198,* 198 (1939).

5. WILLIAMS, R. D., H. L. Mason, R. M. Wilder and B. F. Smith. Induced vitamin B_1 deficiency in human subjects. *Proc. Staff. Meet., Mayo Clinic 14,* 787 (1939).

6. WILLIAMS, R. D., H. L. Mason and R. M. Wilder. Observations on induced thiamine (vitamin B_1) deficiency in man. *Arch. Int. Med. 66,* 785 (1940).

7. WILLIAMS, R. D., H. L. Mason. Further observations on induced thiamine (vitamin B_1) deficiency and thiamine requirements of man. *Proc. Staff. Meet., Mayo Clinic 16,* 433 (1941).

8. WILLIAMS, R. D., H. L. Mason, B. F. Smith and R. M. Wilder. Induced thiamine (vitamin B_1) deficiency and the thiamine requirement of man. *Arch. Int. Med. 69,* 721 (1942).

9. WILLIAMS, R. D., H. L. Mason, M. H. Power and R. M. Wilder. Induced Thiamine (Vitamin B_1) Deficiency in Man. *Arch. Internal Med. 71,* 38 (1943).

10. NAJJAR, V. A. and L. E. Holt. The biosynthesis of thiamine in man and its implications in human nutrition. *J. Am. Med. Assoc. 123,* 683 (1943).

11. NAJJAR, V. A., G. A. Johns, G. C. Medairy, G. Fleishmann and L. E. Holt, Jr. The Biosynthesis of Riboflavin in Man. *J. Am. Med. Assoc. 126,* 357 (1944).

12. BUEDING, E., M. H. Stein and H. Wortis. Xanthopterin, the fish anemia factor. *J. Biol. Chem. 140,* 679 (1941).

13. GOLDBERGER, J., G. A. Wheeler and E. Sydenstricker. A study of the diet of non-pellagrous and of pellagrous households in textile mill communities in South Carolina in 1916. *J. Am. Med. Assoc. 71,* 944 (1918).

14. SEBRELL, W. H. Vitamins in relation to the prevention and treatment of pellagra. *J. Am. Med. Assoc. 110,* 1665 (1938).

15. SEBRELL, W. H. and R. E. Butler. Riboflavin deficiency in man. A preliminary note. *U. S. Pub. Health Rpt. 53,* 2282 (1938).

16. SEBRELL, W. H. and R. E. Butler. Riboflavin deficiency in man. (Ariboflavinosis). *U. S. Pub. Health Rpt. 54,* 2121 (1939).

17. SEBRELL, W. H., R. E. Butler, J. G. Woolley and H. Isbell. Human riboflavin requirement estimated by urinary excretion of subjects on controlled intake. *U. S. Pub. Health Rpt. 56,* 510 (1941).

18. SYDENSTRICKER, V. P., L. E. Geeslin, C. N. Templeton and J. W. Weaver. Riboflavin deficiency in human subjects. *J. Am. Med. Assoc. 113,* 1697 (1939).

19. JOLLIFFE, N., H. D. Fein and L. A. Rosenblum. Riboflavin deficiency in man. *New Eng. J. Med. 221,* 921 (1939).

20. KRUSE, H. D., V. P. Sydenstricker, W. H. Sebrell and H. M. Cleckley. Ocular manifestations of ariboflavinosis. *U. S. Pub. Health Rpt. 55,* 157 (1940).

21. SPIES, T. D., W. B. Bean, R. W. Vilter and N. E. Huff. Endemic riboflavin deficiency in infants and children. *Am. J. Med. Sci. 200,* 697 (1940).

22. ODEN, J. W., L. H. Oden and W. H. Sebrell. Report of three cases of ariboflavinosis. *U. S. Pub. Health Rpt. 54,* 790 (1939).

23. SPIES, T. D., R. W. Vilter and W. F. Ashe. Protein Extracts — Arlco. *J. Am. Med. Assoc. 113,* 939 (1939).

24. BARBORKA, C., E. Foltz and A. C. Ivy. Relationship between vitamin B complex intake and work output in trained subjects. *J. Am. Med. Assoc. 122,* 717 (1943).

25. EGANA, R. E. Johnson, R. Bloomfield. The effects of a diet deficiency in the vitamin B complex on sedentary men. *Am. J. Physiol. 137,* 731 (1941).

26. KEYS, A. and A. F. Henschel. Vitamin supplementation of U. S. Army rations in relation to fatigue and the ability to do muscular work. *J. Nutr. 23,* 259 (1942).

27. KEYS, A., A. F. Henschel, O. Mickelsen and J. M. Brozek. The performance of normal young men on controlled thiamine intakes. *J. Nutr. 26,* 399 (1943).

28. KEYS, A., A. F. Henschel, O. Mickelsen, J. M. Brozek and J. H. Crawford. Physiological and biochemical functions in normal young men on a diet restricted in riboflavin. *J. Nutr. 27,* 165 (1944).

29. KEYS, A., A. Henschel, H. L. Taylor, O. Mickelsen and J. Brozek. Absence of rapid deterioration in men doing hard physical work on a restricted intake of vitamins of the B complex. *J. Nutr. 27,* 485 (1944).

30. KEYS, A., A. Henschel, H. L. Taylor, O. Mickelsen and J. Brozek. Experimental studies on man with a restricted intake of the B vitamins. *Am. J. Physiol. 144,* 5 (1945).

31. JOHNSON, R. E., R. C. Darling, W. H. Forbes, L. Brouha, E. Egaña and A. Graybiel. The effects of a diet deficient in part of the vitamin B complex upon men doing manual labor. *J. Nutr. 24,* 585 (1942).